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Nanoscale protein patterning on Si substrates using colloidal lithography and plasma processing

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Abstract

Selective immobilization of proteins in well-defined patterns on substrates has recently attracted considerable attention as an enabling technology for applications ranging from basic research to biosensors and bioMEMS. In this work we demonstrate a low-cost and high throughput process for nanoscale, selective immobilization of proteins on patterned Si substrates, based on colloidal lithography and plasma processing in order to define the spots (< 300 nm) where proteins are adsorbed. A close-packed monolayer of PS microparticles is deposited on oxidized Si substrates and is used as etching mask to define SiO_2 spots (on Si), which after plasma-induced chemical modification are appropriate for selective protein immobilization. The immobilized proteins are detected by means of confocal microscopy and evaluated by means of atomic force microscopy. Such nanoscale immobilized proteins can be successfully integrated into BioMEMS and microanalytical systems.

Keywords: Colloidal lithography; plasma treatment ; protein patterning; BioMEMS

1. Introduction

The potential of proteins to be integrated into micro or nanofabricated devices is steadily gaining importance for applications such as biosensors, bioMEMS, tissue engineering, as well as protein arrays [1-2]. To create protein patterns, photolithography-based methods have been extensively used. However

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for features smaller than 1 μm, photolithography using contact or proximity printing is reaching its limits, and becomes non cost-efficient. Dip-pen and e-beam lithography which can successfully achieve nanoscale protein patterning, they usually involve complex and high cost apparati, are serial processes and, as such, lack scalability.

In this work, we propose a low-cost and high-throughput method for very high density protein patterning, based on colloidal lithography [3-4] and plasma processing [5] to define the spots where proteins are selectively adsorbed. The advantages of our method include simplicity and time-saving experimental procedures due to the plasma-assisted selective chemical modification of the surface, compared to other functionalization protocols based on wet chemistries [6].

2. Substrate preparation & protein deposition process

The fabrication procedure is described schematically in Fig.1. A close-packed monolayer of PS microparticles is deposited on oxidized Si substrates and a short O_2 plasma step is used for microparticle size reduction to about 300 nm or less, depending on the colloidal microparticle initial size. Subsequently, the shrunk colloidal particles are used as etching masks to define SiO_2 spots on Si substrate using C_4F_8 plasma to etch the SiO_2 in the areas between the particles. Subsequently, the PS microparticles are removed from the surface and a short selective chemical modification of the substrate is performed in C_4F_8 plasma under optimized conditions [7]. The latter is a treatment appropriate for selective protein adsorption on SiO_2 spots versus Si areas. After substrate preparation, the protein deposition follows. In particular, biotinylated Bovine Serum Albumin (BSA) is deposited and after incubation, a blocking step is performed using a buffer phosphate solution containing BSA (10 g/l BSA solution in 50mM phosphate buffer pH 7.4) in order to cover the remaining free protein binding sites of the surface. The immobilized protein can be detected after reaction with the fluorescently-labeled streptavidin (AF546-labeled streptavidin, 5μg/ml solution).

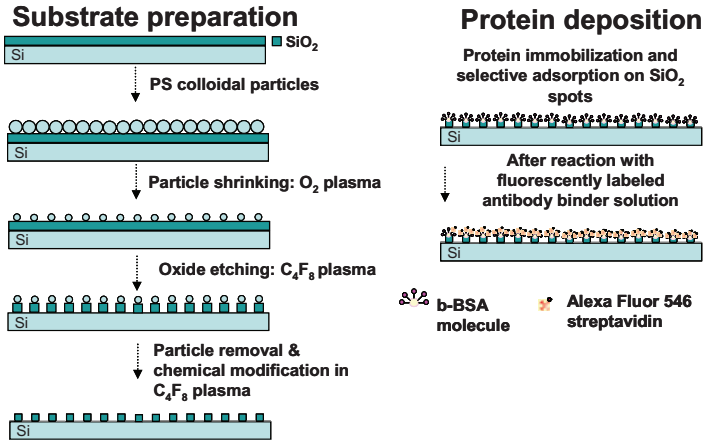
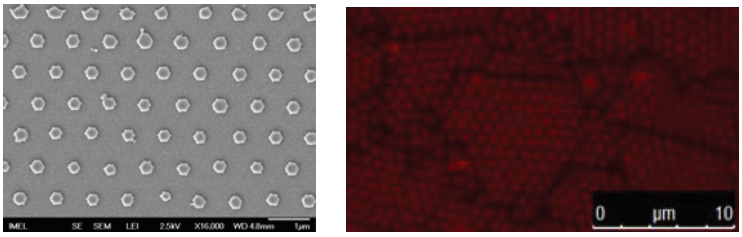


Fig.1. Schematic of the preparation of nanopatterned SiO_2/Si substrates and the protein immobilization process

3. Results & Discussion

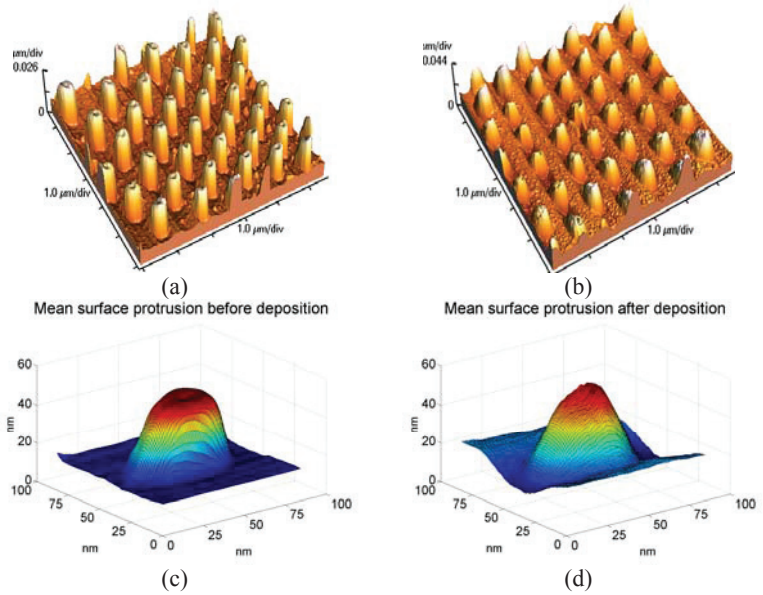
In order to evaluate our prepared substrates before protein deposition, Scanning Electron Microscopy (SEM) images were obtained. The image in Fig. 2 (a) confirms the formation of SiO_2 spots (300 nm in

diameter) on the Si substrate. To check for selective protein immobilization, fluorescence images were obtained by means of a confocal microscope. The image in Fig. 2 (b) demonstrates selective protein immobilization on the SiO_2 spots, where the fluorescently labeled streptavidin has been bound to adsorbed biotinylated BSA in contrast to the dark (Si) areas around the spots. In order to evaluate the thickness of the protein layer adsorbed on the SiO_2 spots, atomic force microscopy, a method widely used in the literature [8] for protein deposition evaluation, was performed, before and after protein adsorption on our nanoscale patterned substrates. A representative AFM image of a patterned and plasma-treated Si substrate is shown in Fig. 3 (a) before and (b) after protein immobilization. Statistical analysis of all spots from at least five such images is performed using a home-made software. This analysis results in an average spot morphology before (Fig. 3c) and after (Fig. 3d) protein immobilization, and from that an average thickness is obtained for the deposited protein layer. From the described experiments and analysis, an average thickness in the range of 3-5 nm for biotinylated BSA is obtained, consistent with its molecular dimensions; 14 nm x 5 nm x 5 nm [9]).



(a) (b)

Fig. 2. (a) SEM image after substrate nanopatterning. (b) Confocal Microscopy fluorescence image after selective protein immobilization on SiO_2 and reaction with AF-546 labeled streptavidin (red spots).



(a) (b) (c) (d)

Fig. 3. AFM images of SiO_2 spots on Si (a) before and (b) after protein immobilization. Statistical analysis of these AFM images estimates an average SiO_2 spot morphology (c) before and (d) after protein deposition, from which an average thickness of the adsorbed protein layer is estimated.

3. Conclusion

The described method of protein nanopatterning based on a combination of colloidal lithography with plasma etching and plasma-induced surface modification constitutes a cost-efficient and rapid route towards very high density patterning of biomolecules on solid supports with potential applications in biosensors, microanalytical systems and cell biology.

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